THE METABOLISM OF STRONTIUM-90 AND CALCIUM-45 BY LEBISTES

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That fishes accumulate mineral elements from the water in which they swim and incorporate these elements into body tissues has recently been demonstrated by the use of calcium-45 and strontium-89 in fresh water and marine fishes of various species (Prosser *et al.,* 1945; Rosenthal, 1956; Lovelace and Podoliak, 1952; Boroughs *et al.,* 1956; Alexander *et al.,* 1956). The rate of incorporation of calcium-45 into the total body and tissues is linear for *Lebistes* and *Salmo* sp. (Rosenthal, 1956; Lovelace and Podoliak, 1952), but bone and osseous tissues incorporate the nuclide at a greater rate than either visceral organs or muscle. In *Lebistes,* the loss of incorporated calcium-45 from the whole body may be described by at least three separate first-order reactions varying from very fast to very slow, probably reflecting the rate of turnover of visceral organs, muscle, and osseous tissues, respectively. In marine fishes, Boroughs *et al.* (1956) have shown that strontium-89, placed in water, is rapidly incorporated into body tissues and the distribution in tissues is similar to that following oral dosage of the nuclide. These investigators also showed that the rate of excretion of a single oral dose of strontium-89 is rapid during the first few days of the experiment. However, the isotope remaining in the body after the first few days persisted at a constant level for a long time.

In view of the reports that small laboratory mammals (Alexander *et al.,* 1956; Comar *et al.,* 1955), man (Turekian and Kulp, 1956) and marine fishes (Boroughs *et al.,* 1957) discriminate against strontium relative to calcium, and since strontium is chemically similar to calcium, it was of interest to determine the uptake and turnover of strontium-90 by *Lebistes* and to compare this information with that previously obtained with calcium-45. The results of this study form the basis for this report.

Materials and Methods

Adult male wild-type guppies, averaging 125 mg. in weight, were obtained from commercial sources. The experimental design and the treatment of animals and tissues for analysis has been described in detail in a previous publication (Rosenthal, 1956).

All samples for radioactivity assay for strontium-90 were counted after a waiting period of 20 days to permit equilibrium between strontium-90 and its yttrium-90 daughter nuclide. The samples were counted with a windowless gas flow counter to less than a 5 per cent statistical error. Corrections for self-absorption of strontium-90 were made when necessary but the 28-year half-life of this nuclide obviated decay corrections. Assay of calcium-45 was performed as previously described, with the same counting assembly.

No attempt was made to differentiate between strontium-90 and its yttrium-90 daughter, and the use of the term strontium-90 throughout this report refers to the combined activities of strontium-90 and yttrium-90 at equilibrium. The efficiency of the counter for strontium-90 and calcium-45 was determined to be 1.5×10^{10} and 1×10^{9} counts per minute per millicurie, respectively. The strontium-90 and calcium-45 were obtained from the Oak Ridge National Laboratories in the form of carrier-free salts.

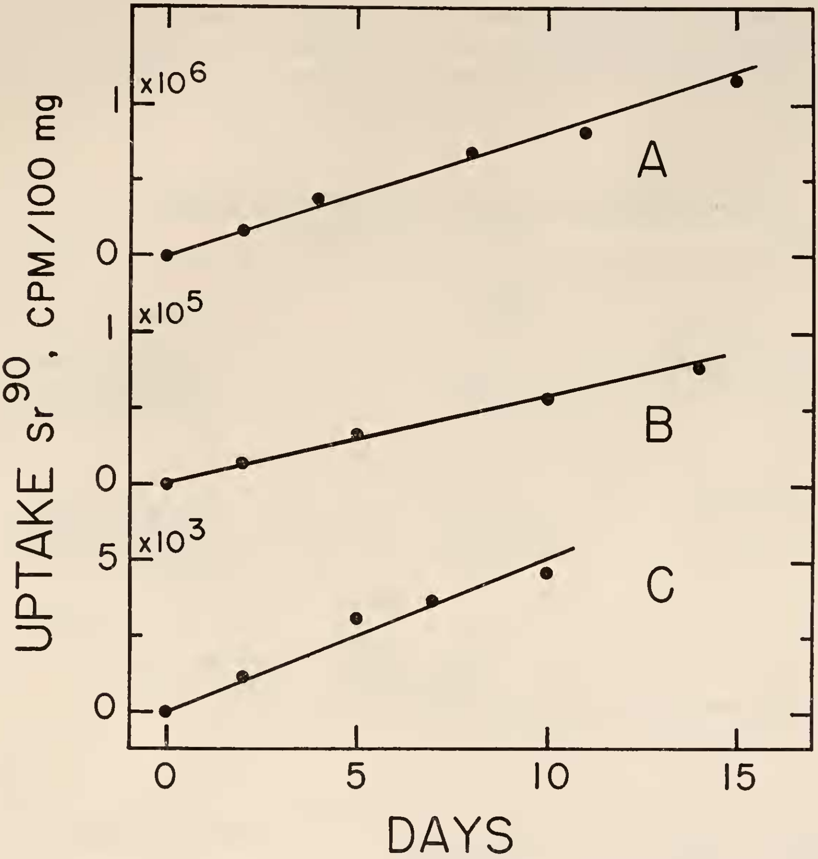

FIGURE 1. Uptake of strontium-90 by male *Lebistes* versus days in water containing the isotope. Each point represents two to four fish. The water activity for Curve A, 1×10^{6} cpm/ml., Curve B, 1.7×10^{5} cpm/ml., Curve C, 1.1×10^{4} cpm/ml.

RESULTS

The rate of uptake of strontium-90 by male *Lebistes* from the water in which they swim was determined by placing the fish in glass aquaria containing 500 milliliters of aged tap water containing the isotope. Distilled water was added daily to compensate for losses of water by evaporation and to maintain the isotope activity of the water within $\pm$ 5 per cent during the experimental period.

The results obtained from these experiments demonstrate the rapid incorporation of strontium-90 into the body of the fish (Fig. 1). The incorporation is linear during a 10- or 15-day experimental period for all concentrations of isotope thus far studied, and the uptake of strontium-90 is similar to the data previously obtained with calcium-45 (Rosenthal, 1956). The similarity between the uptake of strontium-90 and calcium-45 is further shown by calculation of a "concentration factor" which relates the logarithm of the rate of incorporation of the isotope in the body of the fish to the logarithm of the activity of isotope in water (Table I). It is apparent that the concentration factors for both calcium-45 and strontium-90 are surprisingly similar within experimental error. These data are markedly different from those obtained for marine fishes by Boroughs *et al.* (1957), who found that *Tilapia* discriminate against strontium-89 relative to calcium. It is conceivable that

Table I

Relationship between rate of uptake and water activity for various nuclides by the body of male Lebistes

Isotope	Water activity cpm/ml.		Concentration factor* ± S.E.
Strontium-90	8.25×10^3		0.70 ± 0.007 (10)**
	1.72×10^5		0.72 ± 0.003 (16)
	1.00×10^6		0.82 ± 0.004 (13)
		Weighted Average	0.75
Calcium-45	8.52×10^3		0.72 ± 0.007 (18)
	9.42×10^4		0.80 ± 0.005 (20)
	7.37×10^5		0.78 ± 0.010 (13)
		Weighted Average	0.77

$$* \text{ Concentration Factor} = \log\left(\frac{\text{Uptake in count/min. per 100 mg. per day}}{\text{Water activity in count/min. per ml.}}\right).$$

** The numbers in parentheses indicate number of animals analyzed.

$$\text{S.E.} = \text{Standard error} = \left(\frac{\Sigma d^2}{n(n-1)}\right)^{\frac{1}{2}}.$$

these differences are due to entirely different mechanisms involving the osmotic physiology of fresh water and marine fishes.

The various organs of the body such as the spine, head, viscera, and muscle also take up strontium-90 in a linear fashion during a 10-day experimental period, but the rate of uptake differs for each organ, as shown in Figure 2. Highly mineralized tissues such as the spine and head accumulate strontium-90 at a greater rate than the total body while soft tissues (muscle and viscera) accumulate less of the isotope. The accumulation of strontium-90 by the tissues of *Lebistes* is qualitatively similar to that of calcium. Although the total body accumulates the same amount of calcium-45 and strontium-90, the ratio of organ isotope concentration to total body isotope concentration for strontium-90 differs significantly from that of calcium-45 in all of the organs studied (Table II). Thus it is apparent that the spine, head

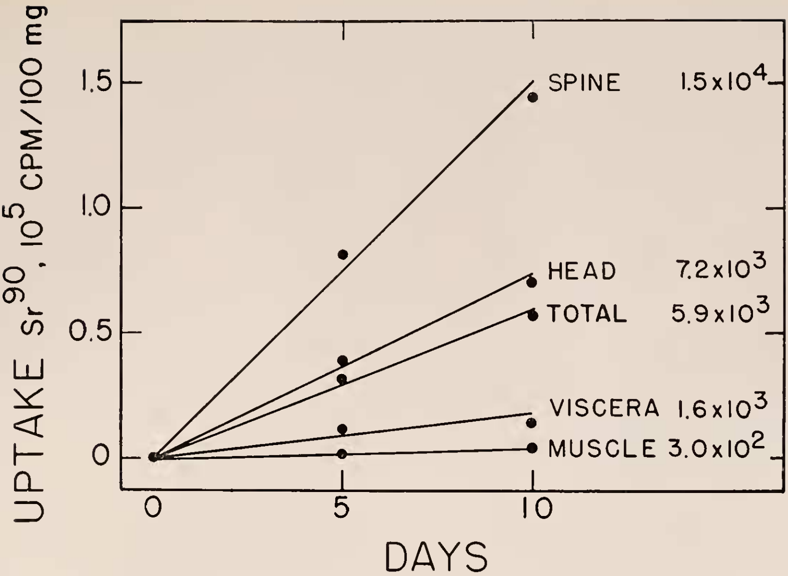

FIGURE 2. Uptake of strontium-90 by various tissues of male *Lebistes* versus days in water containing 9×10^4 counts per minute per milliliter. Values for each tissue represent rate of uptake of strontium-90 in terms of counts per minute per 100 milligrams per day. Each point represents 6 values.

and viscera accumulate significantly more strontium-90 than calcium-45 on a concentration basis. Muscle tissue, on the other hand, tends to incorporate somewhat less strontium-90 than calcium-45. A comparison of the total distribution of strontium-90 and calcium-45 in the various tissues of the body following 10 days of uptake of the isotope from water (Table III) is consistent with the data based on

TABLE II

Relative uptake of strontium-90 and calcium-45 by tissues of male Lebistes

Tissue	Strontium-90*	Calcium-45*	"t"	"P"
Carcass	1.00 ± 0.026** (16)***	1.00 ± 0.045** (20)	—	—
Head	1.28 ± 0.051 (18)	1.07 ± 0.039 (19)	3.29	<0.01
Viscera	0.96 ± 0.118 (18)	0.59 ± 0.087 (21)	2.49	<0.02
Muscle	0.061 ± 0.020 (17)	0.102 ± 0.024 (20)	3.95	<0.01
Spine	2.28 ± 0.10 (15)	1.87 ± 0.10 (16)	3.62	<0.01

* The values represent the ratio $\frac{\text{cpm/100 mg. tissue}}{\text{cpm/100 mg. carcass}}$ derived from 3 to 5 experiments in which the water activity varied from 10^4 to 10^6 cpm/ml. for each isotope.

** Standard error.

*** The numbers in parentheses indicate number of fish analyzed.

$$\text{Students ``t'' value} = \left[\frac{M_1 - M_2}{\left(\frac{\Sigma d_1^2 + \Sigma d_2^2}{N_1 + N_2 - 2}\right)^{\frac{1}{2}}}\right]\left[\left(\frac{N_1 N_2}{N_1 + N_2}\right)^{\frac{1}{2}}\right].$$

TABLE III

Distribution of strontium-90 and calcium-45 in tissues of male Lebistes after 10 days in isotopic water

Tissue	Isotope distribution ± S.E. (per cent of total)		"t"	"P"
	Strontium-90	Calcium-45†		
Carcass	100.0 ± 10.11 (13)*	100.0 ± 2.92 (15)*	—	—
Head	29.9 ± 0.97 (13)	21.3 ± 1.12 (14)	5.71	<0.01
Viscera	13.5 ± 1.87 (13)	7.3 ± 0.48 (14)	3.34	<0.01
Muscle***	2.8 ± 0.29 (12)	3.7 ± 0.31 (14)	2.12	<0.05
Spine	6.9 ± 0.61 (12)	6.2 ± 0.36 (14)	1.02	—
Remainder**	46.9 ± 2.24 (12)	61.5 ± 2.32 (13)	4.36	<0.01

* The numbers in parentheses indicate number of animals analyzed.
** Calculated by difference.
*** Muscle tissues estimated to comprise 40 per cent of body weight.
† From Rosenthal (1956).

concentration shown in Table II. The apparent discrepancy for the similarity between the distribution of calcium-45 and strontium-90 in the spine (Table III) and the relative uptake of the two isotopes by the spine on a concentration basis is due, in all probability, to our inability to always remove the entire spine from these small fishes. This unavoidable error introduces some uncertainty into the distribution data for the spines.

The head and "remainder" (skin, scales and fins) account for 77 per cent of the total body strontium-90, while these tissues account for almost 83 per cent of the total body calcium-45. Muscle contains the smallest proportion of the total body strontium and calcium (2.8 and 3.7 per cent, respectively) while occupying about 40 per cent of the total body weight. The spine, representing less than 3 per cent of the body weight, contains between 6 and 7 per cent of the total calcium and strontium nuclides, respectively. This comparison between the distribution of strontium-90 and calcium-45, under the same experimental conditions, indicates that the head and viscera incorporate, respectively, 25 per cent and 46 per cent more strontium-90 than calcium-45, while muscle and the remaining tissues accu-

TABLE IV

Relative proportions of tissues of male Lebistes

Tissue	No. of determinations	Per cent of body weight
Head	39	20.6 ± 0.22*
Viscera	38	12.3 ± 0.37
Spine	21	2.8 ± 0.07
Remainder**	39	24.4 ± 0.25
Muscle	—	40.0 (estimated)

* Standard error.
** Remainder includes skin, fins, scales and is calculated by difference assuming that muscle comprises 40 per cent of body weight.

mulate about 25 per cent less strontium-90 than calcium-45. These differences in tissue uptake of the two nuclides are statistically significant and further accentuate subtle differences in the metabolism of these two elements.

Since the relationship of the weight of organs to body weight has not, to my knowledge, been previously determined or published, for *Lebistes*, the relative proportions of the various tissues analyzed in this study are shown in Table IV.

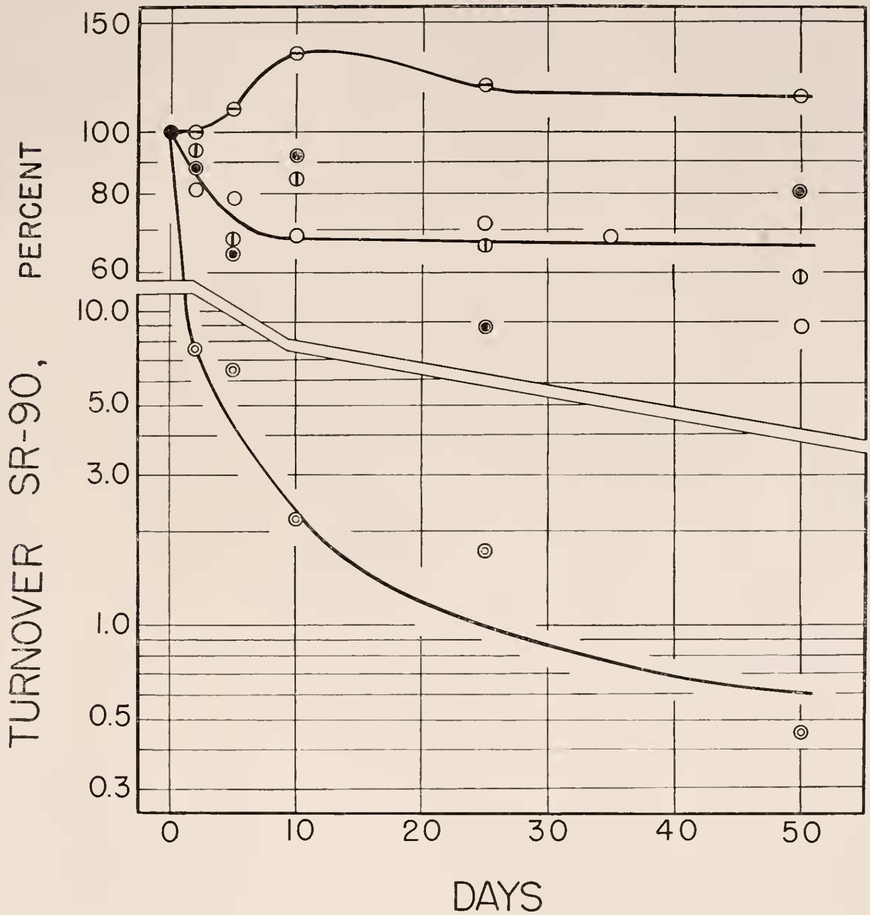

FIGURE 3. Turnover of strontium-90 by various tissues of male *Lebistes* versus days in water containing no isotope. Each point represents 4 to 11 values obtained from 3 experiments. ⊖, spine: ○, body: ●, muscle: ⦶, head: ◎, viscera. The fish contained about 10^4 cpm/100 mg. on day zero of turnover.

The rate of turnover of strontium-90 by the body and tissues of *Lebistes* was determined by first placing the animals in isotope-containing water for 10 days in order to incorporate sufficient radioactivity into the tissues. After this period the fishes were transferred to isotope-free water which was changed periodically and they were sacrificed at suitable intervals previously described in detail (Rosenthal, 1956). During a 50-day experimental period, the loss of strontium-90 from the total fish could be resolved into two components that may be described by first order reactions (Fig. 3). The first component, turning over rapidly with a biological half-life of about 8 days, represents loosely-bound strontium-90 in visceral tissues of the body. This is somewhat longer than the three-day half-life for the rate of turnover of the fast component with calcium-45 (Rosenthal, 1956). The loss of strontium-90 by the viscera is extremely rapid, so that 92 per cent of the radioactivity is lost during the first two days of the experiment. The second component has an exceedingly long half-life of about two years or more. During a similar experimental period with calcium-45 (Rosenthal, 1956) three components with biological half-lives of 3 days, 137 days, and 309 days were apparent which reflect the turnover rates of visceral tissues, muscle and carcass, respectively. The absence of an intermediate component for strontium-90 is primarily due to the very slow turnover rate of strontium-90 by muscle tissue, and to a lesser extent, the head.

TABLE V

Distribution of strontium-90 and calcium-45 in tissues of male Lebistes after 40–50 days of turnover in non-isotopic water

Tissue	Isotope distribution ± S.E. (per cent of total)		"t"	"P"
	Strontium-90 (50 days)	Calcium-45 (40 days)†		
Carcass	100.0 ± 11.2 (5)*	100.0 ± 5.48 (8)*	—	—
Head	42.7 ± 5.16 (5)	34.4 ± 2.08 (7)	1.69	>0.15
Viscera	0.2 ± 0.06 (5)	0.5 ± 0.04 (5)	2.12	>0.05
Muscle***	3.0 ± 0.87 (5)	2.6 ± 0.46 (5)	0.56	NS
Spine	21.0 ± 4.47 (5)	19.4 ± 1.04 (7)	0.41	NS
Remainder**	33.1 ± 7.79 (5)	43.1 ± 2.38 (6)	1.33	>0.20

* The numbers in parentheses indicate number of animals analyzed.
** Calculated by difference.
*** Muscle tissue estimated to comprise 40 per cent of body weight.
† From Rosenthal (1956).

It is of interest to note that muscle tissue strontium-90 with a biological half-life of about two years as calculated from the last 25 days of the experiment, is lost in a manner similar to that of the total body. This is in contrast to the biological half-life for muscle of 137 days as determined previously for calcium-45, and it would appear that the metabolism of the two elements differs in muscle tissue. The exceptionally slow turnover of strontium-90 in muscle tissue of marine fishes has recently been observed by Boroughs *et al.* (1956).

The spine, which consists not only of mineral matter but also of intervertebral cartilage, tendon, and organic bone matrix, continues to incorporate the isotope for about 10 days after the fish is placed in isotope-free water. The additional nuclide must be derived from a redistribution of isotope from soft tissues such as viscera. Similar data were obtained with calcium-45 (Rosenthal, 1956). The accumulated isotope does not remain fixed in the spine, however, but is subsequently lost and a new equilibrium consistent with that of the mineral component of bone becomes established. The biological half-life of strontium-90 in the spine, calculated during the last 25 days of the experiment, may be estimated to exceed two years, a value consistent with the biological half-life of calcium-45 previously determined (Rosenthal, 1956). The additional increase of the strontium-90 and calcium-45 in the bone and its relatively rapid loss may represent a rather labile binding-site ($T\frac{1}{2}$ = about 50 days) for bone formation.

The distribution of strontium-90 in various organs and tissues of the body after 50 days in isotope-free water is compared with the distribution of calcium-45 after 40 days in isotope-free water (Table V). It is interesting to note that the distributions of both isotopes at the end of 40 days of turnover for calcium-45 and 50 days for strontium-90 are not significantly different. A comparison of the rate of turnover of strontium-90 by the tissues, shown in Figure 3 of this report, with the rate of turnover of calcium-45 previously published (Rosenthal, 1956) indicates that the similarity of distribution of both isotopes at these particular time intervals is coincidental. Extrapolation of the turnover rates for both isotopes indicates that the head, muscle and spine would retain a greater proportion of the body strontium-90, while the viscera and "remainder" would contain less strontium-90 throughout the life of the fish.

Discussion

It is apparent from these studies that fresh water fishes accumulate strontium-90 from the water in which they swim and that the rate of uptake is similar to that of calcium-45. Moreover, we have recently shown that the rate of uptake of strontium-90 and calcium-45 by other fresh water fishes (*Danio* and *Tanichthys*) is similar to the data we have obtained with *Lebistes*. These data differ from the studies of Boroughs *et al.* (1957), who found that marine fishes discriminate against strontium-89. This apparent disagreement may be due to marked differences in osmotic regulation between marine and fresh water fishes. On the other hand, discrimination of strontium isotopes relative to calcium by small laboratory mammals appears to be well documented (Comar *et al.*, 1955; Turekian and Kulp, 1956; Comar *et al.*, 1956). Comar *et al.*, (1956) have indicated that the processes of major discrimination, in rats, are decreased absorption of strontium from the intestinal tract and increased urinary strontium excretion, processes which cannot be measured directly in small fishes. These two processes would tend to limit the quantity of strontium entering the body and its retention in the body, but in fishes, the gills play a major role for the absorption and excretion of mineral elements. The similarity of the "concentration factor" (Table I) for both nuclides by the total body of the fish indicates no discrimination of strontium-90 by these fishes. It is possible that subtle differences between strontium-90 and calcium-45 uptake by fishes may become apparent by the use of differential methods in which both nuclides are present in the same medium. These and other aspects of the problem are under investigation.

The rate of excretion of strontium-90 by the total body and tissues (except viscera) of *Lebistes* is slower than that of calcium-45. The data appear to be in contrast with the report by Lengeman (1957), who showed that rat bones, *in vitro*, lose more strontium-90 than calcium-45. Since the accumulation and retention of mineral elements into bone depend on the rate of bone formation and sequestration of elements into slowly exchanging bone matrix, comparisons between such diverse biological systems may be hazardous. Nonetheless, tissues of *Lebistes* rich in calcium, such as the spine and head, accumulate and retain a larger percentage of the total body strontium-90 than that of calcium-45, in accord with the studies of Lengeman (1957) and Comar *et al.* (1956) for rats. Although no explanation is offered at this time concerning the mechanism of incorporation of alkaline earth elements from water by fishes, the similarity of uptake of calcium-45 and strontium-90 by fresh water fishes (Rosenthal, 1957) indicates a fundamental and essentially similar process.

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Summary

1. The uptake of strontium-90 by male *Lebistes* from the water in which they swim is linear with time for the total carcass and tissues studied. Tissues containing high concentrations of calcium accumulate more strontium-90 than soft tissues. The rate of turnover of the nuclide varies from very fast to very slow according to the type of tissue. The whole body, head and spine retain strontium-90 for long periods of time ($T\frac{1}{2} = 600$ days) while viscera loses the isotope rapidly ($T\frac{1}{2} = 8$ days).

2. A comparison between strontium-90 and calcium-45 uptake and turnover by male *Lebistes* are qualitatively similar but significant quantitative differences are apparent.

LITERATURE CITED

Alexander, G. V., R. E. Nusbaum and N. S. MacDonald, 1956. The relative retention of strontium and calcium in bone tissue. *J. Biol. Chem.*, **218**: 911–919.

Boroughs, H., S. J. Townsley and R. W. Hiatt, 1956. The metabolism of radionuclides by marine organisms. I. The uptake, accumulation, and loss of strontium[89] by fishes. *Biol. Bull.*, **111**: 336–351.

Boroughs, H., S. J. Townsley and R. W. Hiatt, 1957. The metabolism of radionuclides by marine organisms. III. The uptake of calcium[45] in solution by marine fish. *Limnology and Oceanography*, **2**: 28–32.

Comar, C. L., I. B. Whitney and F. W. Lengeman, 1955. Comparative utilization of dietary strontium-90 and calcium by developing rat fetus and growing rat. *Proc. Soc. Exper. Biol. Med.*, **88**: 232–236.

Comar, C. L., R. H. Wasserman and M. M. Nold, 1956. Strontium-calcium discrimination factors in the rat. *Proc. Soc. Exper. Biol. Med.*, **92**: 859–863.

Lengeman, F. W., 1957. Comparative metabolism of strontium-89 and calcium-45 by bone grown *in vitro*. *Proc. Soc. Exper. Biol. Med.*, **94**: 64–66.

Lovelace, F. E., and H. A. Podoliak, 1952. Absorption of radioactive calcium by brook trout. *Prog. Fish. Cult.*, **14** (4): 154–158.

Prosser, C. L., W. Pervinsek, J. Arnold, G. Svihla and P. C. Tompkins, 1945. Accumulation and distribution of radioactive strontium, barium-lantharnum, fission mixture and sodium in goldfish. *U. S. Atomic Energy Comm. Tech. Inform. Serv. MDDC.-496.*

Rosenthal, H. L., 1956. Uptake and turnover of calcium-45 by the guppy. *Science*, **124**: 571–574.

Rosenthal, H. L., 1957. Uptake of calcium-45 and strontium-90 from water by freshwater fishes. *Science*, **126**: 699–700.

Turekian, K. K., and J. L. Kulp, 1956. Strontium content of human bones. *Science*, **124**: 405–406.